

# Synthesis of Chondroitin Sulfate A Bearing Syndecan-1 Glycopeptide

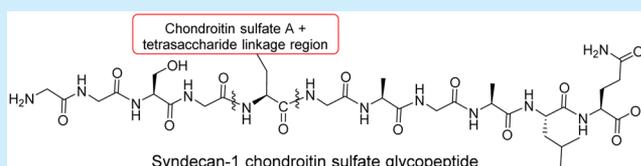
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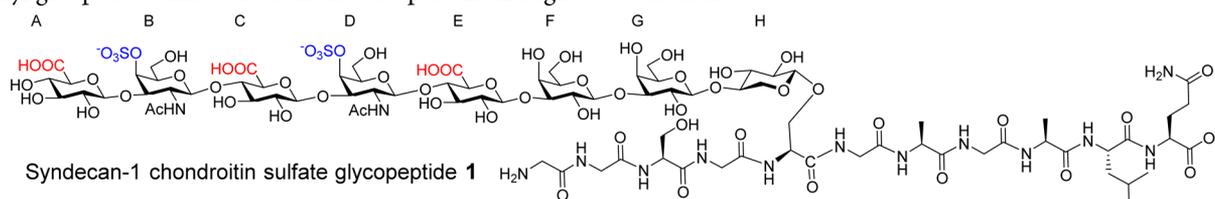
**S** Supporting Information

**ABSTRACT:** Syndecan-1 chondroitin sulfate glycopeptide was synthesized for the first time using the cassette approach. The sequence of glycosylation to form the octasaccharide serine cassette was critical. The glycopeptide was successfully assembled via a 2+ (3 + 3) glycosylation strategy followed by peptide chain elongation.



Chondroitin sulfates (CSs) are polyanionic complex polysaccharides consisting of a disaccharide repeating unit of D-glucuronic acid (GlcA) β-1,3-linked to 2-acetamido-2-deoxy-D-galactosamine (GalNAc). CSs belong to the glycosaminoglycan family and exist as a part of chondroitin sulfate proteoglycans (CSPGs) in nature. In CSPG, CSs are covalently linked to the hydroxyl group of a serine residue in the core protein through a

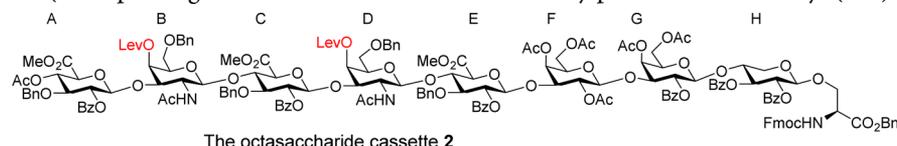
tetrasaccharide linker of GlcA-β-1,3-galactose (Gal)-β-1,3-Gal-β-1,4-xylose.<sup>1–3</sup> A representative example of CSPG is syndecan-1, which interacts with a large variety of biological molecules such as growth factors, chemokines, amyloid β, and cell adhesion molecules.<sup>1–6</sup> As a result, it plays important roles in mediating cell binding, signaling, proliferation, and cytoskeletal organization.



CS and CSPG on average contain one O-sulfate per disaccharide unit, which is installed by O-sulfotransferases in nature.<sup>7</sup> The major types of CS have a 4- or 6-O-sulfo group on the GalNAc residue, which are referred to as CS-A and CS-C, respectively. Other sulfate variants are also known.<sup>1,3,8</sup> Enzymatic sulfation reactions are often not complete, leading to high structural heterogeneities of naturally existing CSs. This significantly hinders the efforts to thoroughly understand the biological functions of CS/CSPG. Although CS oligosaccharides<sup>8–10</sup> and the tetrasaccharide linkage region<sup>11</sup> have been prepared, to date, the synthesis of CSPG glycopeptide bearing a homogeneous CS glycan chain has not been achieved. This paper addresses this critical need by providing a synthesis of the CSPG syndecan-1<sup>12</sup> glycopeptide **1**, which bears a CS-A tetrasaccharide on a core protein sequence (corresponding to amino acid residues

45–53 of human syndecan-1) with two additional glycine residues at its N-terminal for future sortase-mediated ligation. In nature, syndecan-1 can bear CS chains on multiple possible serine locations. A successful completion of the synthesis of CSPG **1** lays important groundwork for the synthesis of other CSPGs.

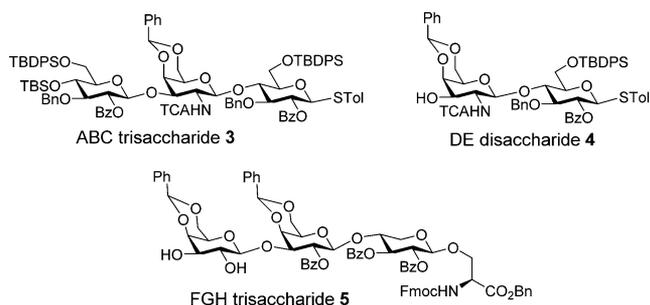
To access CSPG glycopeptide **1**, a cassette approach<sup>13</sup> was chosen, in which the glycosyl amino acid would be prepared as a cassette for peptide elongation because although a short peptide can be glycosylated chemically,<sup>14</sup> it is often challenging to directly glycosylate a large peptide acceptor. CS-A octasaccharide-linked serine **2**, containing the full tetrasaccharide linkage region, galactosamine (GalN) β-linked GlcA, and O-sulfation, was designed as a cassette with the two sites for O-sulfation selectively protected as levulinoyl (Lev) esters.



With the large size of the glycan chain in **2**, there are many potential reaction sequences for glyco-assembly. The first route investigated was through 3 + 2 + 3 glycosylation<sup>15</sup> to access the

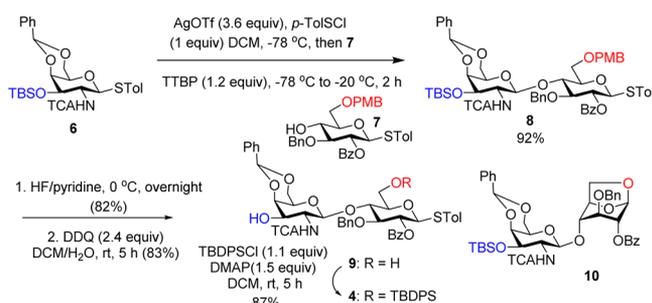
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octasaccharide with strategic disconnections at C/D and E/F linkages. Whereas most published CS syntheses take advantage of glycosyl trichloroacetimidates as building blocks,<sup>8–10</sup> thioglycosides can be an attractive alternative due to their higher stabilities.<sup>16</sup> The amine moiety of GalN was protected as a trichloroacetyl (TCA) amide, which could direct  $\beta$  stereoselectivity<sup>8–10</sup> and reduce the potential complications from the use of acetamide bearing building blocks.<sup>17,18</sup> Thus, ABC trisaccharide 3, DE disaccharide 4, and FGH trisaccharide 5<sup>19</sup> were designed first to access octasaccharide 2.



Synthesis started with preparation of the DE disaccharide 4 by a glycosylation reaction between donor 6 and acceptor 7 (Scheme 1). Donor 6 was preactivated with *p*-TolSCI/AgOTf<sup>20</sup>

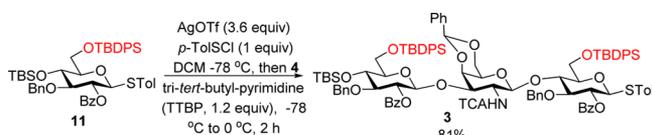
#### Scheme 1. Preparation of DE Disaccharide 4



at  $-78$  °C. Upon complete activation of donor (5 min), acceptor 7 was added together with a non-nucleophilic base tri-*t*-butylpyrimidine.<sup>21</sup> Disaccharide 8 was obtained in an excellent yield of 92%. Protective group manipulation of 8 gave TBDPS-protected disaccharide 4. It was necessary to switch the PMB in 8 to TBDPS, as activation of 8 as a glycosyl donor in a subsequent glycosylation reaction gave the 1,6-anhydrosugar 10 as a major side product. The TBDPS group in 4 was chosen because it can be selectively removed and the resulting free primary hydroxyl groups can be oxidized after glyco-assembly to generate the corresponding GlcA.

To prepare the ABC trisaccharide (Scheme 2), glucoside donor 11 was preactivated for 5 min at  $-78$  °C, and then disaccharide acceptor 4 was added over 2 min in DCM to generate the ABC trisaccharide 3 in 81% yield. Formation of the CD glycosyl linkage was tested next. However, glycosylation of donor 3 and disaccharide acceptor 4 gave very low yield (<10%) of

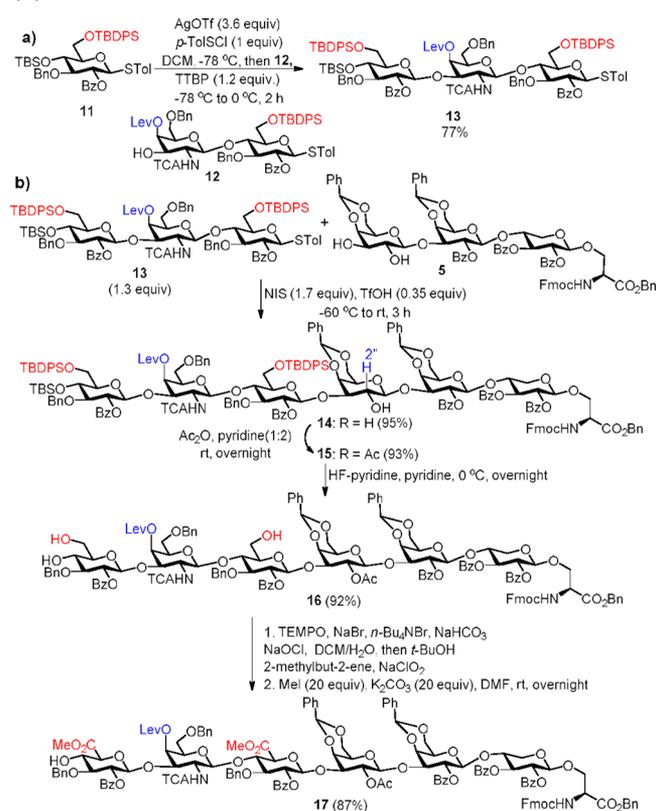
#### Scheme 2. Preparation of ABC Trisaccharide Donor 3



the desired pentasaccharide. Extensive efforts in varying the protective groups on either 3 or 4 failed to improve the glycosylation yield. It should be noted that both AB and CD linkages are between glucosyl and GalN units. The much higher glycosylation yield in using donor 11 to glycosylate disaccharide 4, compared with the reaction between 3 and 4, suggests that it is difficult to use a trisaccharide donor to form the CD linkage, presumably due to the reduced reactivity of an oligosaccharide glycosyl donor (e.g., 3) vs the corresponding monosaccharide (e.g., 11).

To overcome these setbacks, we designed a new 2 + (3 + 3) synthetic route, wherein the difficult CD linkage was formed earlier in the synthesis. Preactivation-based glycosylation of glucosyl donor 11 and disaccharide 12 gave the CDE trisaccharide 13 in 77% yield (Scheme 3a). Glycosylation between

#### Scheme 3. Synthesis of (a) CDE Trisaccharide Donor 13 and (b) Hexasaccharide 17

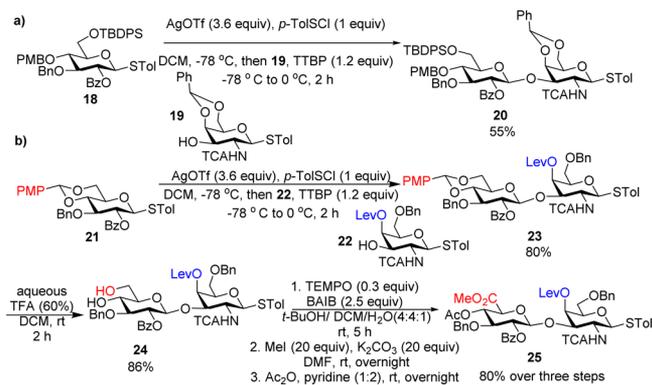


donor 13 and FGH trisaccharide acceptor 5,<sup>19</sup> promoted by *N*-iodosuccinimide/TfOH, proceeded smoothly (Scheme 3b), forming the CDEFGH hexasaccharide 14 in an excellent yield (95%). The free hydroxyl group at C2 on hexasaccharide 14 was acetylated with Ac<sub>2</sub>O to produce 15, which was treated with HF/pyridine to form the triol hexasaccharide 16 in 92% yield. The regiostereoselectivity in the glycosylation of 13 and 5 was confirmed by comparing the chemical shift difference of H2' in 14 vs 15. Selective oxidation of the two primary hydroxyl groups in triol 16 was tested first using TEMPO and [bis(acetoxy)-iodo]benzene.<sup>22</sup> However, this reaction was slow, with multiple partial oxidation side products presumably due to the slow hydration of the aldehyde intermediates. Similar phenomena have been observed in synthesis of a *Staphylococcus aureus* associated trisaccharide.<sup>23</sup> Switching the oxidation protocol to a two-step, one-pot oxidation (TEMPO, NaOCl followed by NaClO<sub>2</sub>)<sup>24</sup>

solved this problem and produced the desired product **17** in 87% yield after methyl ester formation.

To access the AB module, donor **18** was preactivated by *p*-TolSCI/AgOTf and then glycosylated acceptor **19**, producing the desired disaccharide **20** in 55% yield (Scheme 4a).

#### Scheme 4. Synthesis of AB Disaccharide Donor

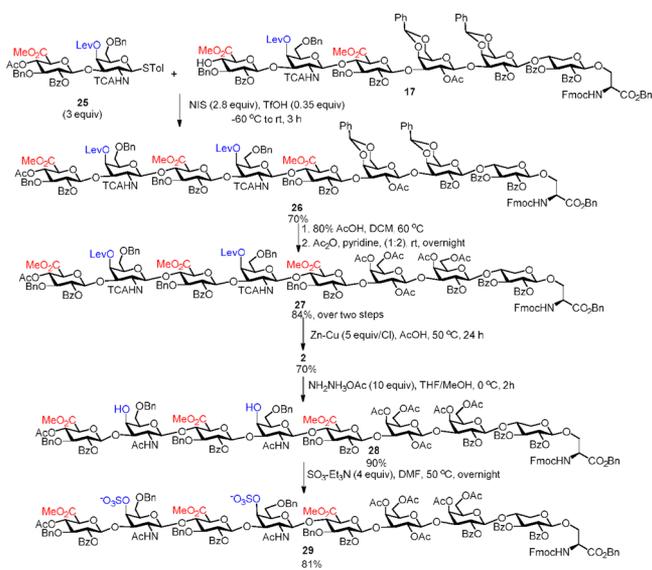


was treated with Et<sub>3</sub>SiH/TFA<sup>25</sup> to selectively open the benzylidene ring for future sulfation, the 4-*O*-PMB group was cleaved. To avoid this complication, we tried the glycosylation of donor **21** with acceptor **22**, which smoothly formed the disaccharide **23** in 80% yield. Cleavage of the *p*-methoxybenzylidene ring in **23** using 60% aqueous TFA led to diol **24**, which then underwent oxidation, methyl esterification, and acetylation, affording the AB disaccharide donor **25** in 80% yield over three steps (Scheme 4b).

With the AB and CDEFGH modules in hand, formation of the octasaccharide was explored. Glycosylation of the hexasaccharide acceptor **17** by disaccharide donor **25** produced octasaccharide **26** in 70% yield (Scheme 5). The successful assembly of **26** indicates that BC and EF linkages are suitable strategic linkage points for constructing the octasaccharide cassette.

The next task was the conversion of the NHTCA in octasaccharide **26** to an acetamide and introduction of the *O*-sulfates. Radical reduction of NHTCA using tributyltin hydride (Bu<sub>3</sub>SnH)/AIBN<sup>26,27</sup> is a popular method that has been successfully applied

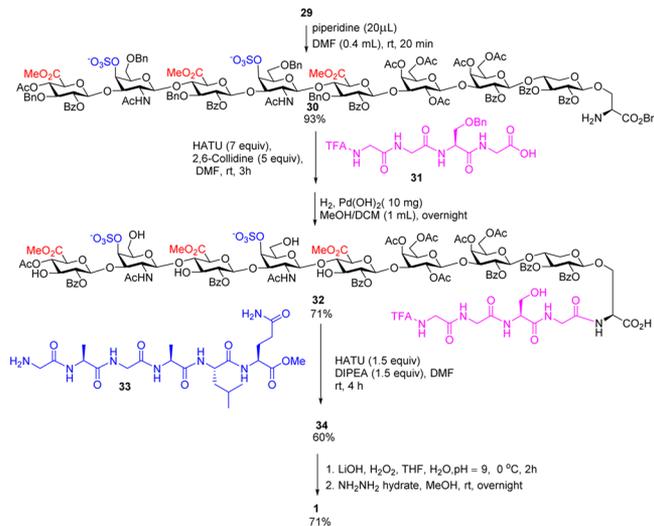
#### Scheme 5. Synthesis of the Octasaccharide Cassette



to CS synthesis.<sup>28,29</sup> However, when **26** was treated with Bu<sub>3</sub>SnH/AIBN, the reaction gave several byproducts containing one or two residual chlorine atoms, which were difficult to separate. As an alternative, we tested the Zn–Cu couple reduction method.<sup>30,31</sup> When octasaccharide **26** was treated with Zn–Cu couple in acetic acid, one or both of the benzylidene rings were cleaved, generating a complex mixture. To overcome this, octasaccharide **26** was first subjected to 80% aqueous acetic acid followed by acetylation of the tetraol, affording octasaccharide **27** (Scheme 5). Subsequent Zn–Cu couple mediated reduction of TCA led to the octasaccharide cassette **2** in 70% yield. The Lev groups in **2** were removed by hydrazine acetate, and the resulting diol **28** was sulfated to provide the octasaccharide **29** in 81% yield, which set the stage for peptide chain elongation.

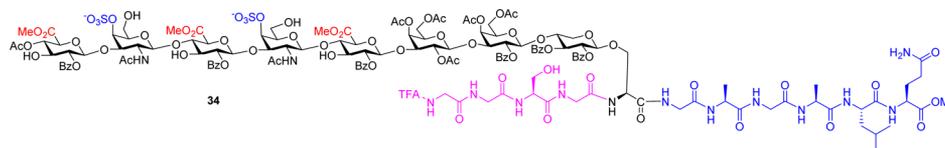
One of the great challenges in CS glycopeptide assembly is the acid sensitivity of *O*-sulfates and the propensities of the glycosyl serine linkages and the glycan chains to undergo  $\beta$ -elimination under basic conditions. Thus, common acid-sensitive amino acid side chain protective groups such as Boc and trityl as well as strongly basic conditions for deprotection should be avoided. The Fmoc group in **29** was removed, and the resulting free amine **30** was coupled with tetrapeptide **31** using *O*-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) (Scheme 6). Subsequent catalytic hydrogenolysis was

#### Scheme 6. Synthesis of Glycopeptide 1



performed with Pearlman's catalyst, which went smoothly to generate the carboxylic acid **32**. Coupling of the carboxylic acid **32** and peptide **31** was promoted by HATU in the presence of diisopropylethyl amine (DIPEA) as the base. During this reaction, we observed a  $\beta$ -elimination side product<sup>32</sup> due to cleavage of glycan from the glycopeptide. The extent of elimination could be reduced by lowering amounts of HATU and DIPEA from 3 equiv typically applied in peptide coupling to 1.5 equiv, leading to the glycopeptide **34** (60% yield).

Deprotection of **34** was first performed using LiOH at pH 9. Even when the reaction was carried out at 0 °C, partial cleavage of the sulfated glycan chain was observed. Switching to the reagent combination of H<sub>2</sub>O<sub>2</sub>/LiOH<sup>28,31,33</sup> at pH 9 avoided the glycan cleavage problem and hydrolyzed the methyl esters as monitored by mass spectrometry. As removal of the benzoyl moieties was very slow under H<sub>2</sub>O<sub>2</sub>/LiOH, hydrazine was found to be a suitable agent to lead to the desired glycopeptide **1** in 71% yield (Scheme 6).



In conclusion, a strategy was successfully established for the first synthesis of a CS glycopeptide. Many obstacles were encountered in the synthesis of this highly complex structure. For glycan chain assembly, we found that it was difficult to form the CD linkage using trisaccharide donors. Rather this linkage was best assembled using a monosaccharide glycosyl donor, which gave the desired product in a high yield. A 2 + (3 + 3) route was developed to successfully prepare the octasaccharide cassette. Subsequently, suitable reaction conditions were identified for peptide chain elongation and deprotection to produce the CS-A bearing syndecan-1 glycopeptide **1**. The successful establishment of a synthetic route to CS glycopeptide will now greatly facilitate understanding of their important biological functions.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.orglett.7b02271](https://doi.org/10.1021/acs.orglett.7b02271).

Experimental procedures and analytical data and NMR spectra of new products (PDF)

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### Notes

The authors declare no competing financial interest.

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